Remarks

The present invention is directed to the construction and expression of fusion enzymes for the production of polymer, where the enzymes are specific bacterial enzymes, and the polymer is polyhydroxyalkanoate. The examples provided in the specification disclose the fusion of the multimeric enzymes requiring the use of cofactors and which interact to synthesize polymer (page 5, lines 21-23).

Independent claim 1 has been amended to clarify that the fusion protein consists of two catalytically active enzymes, which are linked together via the carboxyl and amino termini. The reference to the promoter has been deleted since it does not form a part of the claimed fusion protein.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-6 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner is respectfully reminded that there is no legal requirement that applicants demonstrated reduction to practice of each and every possible member of the claimed species. Applicants are only required to provide one of ordinary skill in the art with enough detail for representative species to demonstrate that they are entitled to the genus. The amount of direction or guidance presented in the specification, in combination with the state of the art at the time of

filing, clearly provides an enabling disclosure for, *inter alia*, any of the genes required to make and use the claimed composition.

The genes encoding all of the claimed proteins are known and publicly available. For example, the Examiner's attention is respectfully drawn to pages 8-10 of the specification, wherein examples of genes encoding PHB and PHA synthases, .beta.-ketothiolases, acyl-CoA reductases, phasins, enoyl-CoA hydratases and .beta.-hydroxyacyl-ACP::coenzyme-A transferases are provided, as well as specific references to journal articles, Medline and GenBank accession numbers disclosing sequences encoding such enzymes (also see pages 4-8 of the Response and Amendment mailed on December 27, 2002). Furthermore, Medline indicates that for each of these classes of enzymes, the amino acid sequence and a cDNA encoding the enzyme are known from multiple sources, and provide evidence that not only is the function generally same between enzymes of different sources, but that the degree of homology is such that the known and available genes can be used to isolate additional genes from other sources encoding the enzymes. If others are desirable, one of ordinary skill in the art may isolate the necessary genes using any of a number of techniques (some of which are described below, in response to the examiner's 35 U.S.C. 112 written description rejection of claims 1-6), including the use of oligonucleotide primers designed to be complementary to the known sequence (and/or degenerate primers) in conjunction with PCR, using the known sequences. Once isolated, construction of gene expression cassettes and transformative plasmids are easily produced.

Applicants note that ***among*** the factors to be considered in determining whether undue experimentation is required (as summarized in *In re* Wands 858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)), are 1) the relative skill of those in the art; and 2) the presence or absence of working examples. The examiner has acknowledged the disclosure of PHA biosynthetic genes on pages 8-11 of the present application and the existence of methods for isolating other genes based on sequence identity to genes with known sequence. These methods (including DNA microarray analysis and functionality assays described below) are all one of ordinary skill would need to acquire any gene harboring sequence that encodes any of the claimed enzymes. "Significant structural *homology*" allows one to classify a gene as encoding a particular class of enzyme. Knowing the *functionality* of an enzyme encoded by a gene will allow one to classify the enzyme as having, for example, a ketothiolase *activity*. Both characteristics are revealed *via* methods commonly used in the art.

It is important to focus on what the claimed invention is, and is not. The claimed invention is a fusion protein of known enzymes, encoded by known nucleic acid sequences. Appropriate expression systems are known. Promoters are described in the application and publicly available. Linkers are described in the application, and publicly available.

The specification discloses many examples of genes that encode β-ketothiolases, acyl-CoA reductases, polyhydroxyalkanoate synthases, poly(3-hydroxybutyrate) synthases, phasins, enoyl-CoA hydratases, and beta-hydroxyacyl-ACP::coenzyme-A transferases. The claimed

fusion constituents, and the genes encoding them, are well known and are well characterized in the art.

In summary, the level of skill in the art is high. It would therefore take no more than routine experimentation for one skilled in the art to make and use the claimed fusion proteins of known enzymes.

Claims 1-6 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Federal Circuit most recently elaborated on the standard under 35 U.S.C. 112 in Enzo Biochem, Inc. v. Gen-Probe (Fed. Cir. July 15, 2002), stating in relevant part:

"It is not correct, however, that all functional descriptions of genetic material fail to meet the written description requirement. The PTO has issued Guidelines governing its internal practice for addressing that issue. The Guidelines, like the Manual of Patent Examining Procedure ("MPEP"), are not binding on this court, but may be given judicial notice to the extent they do not conflict with the statute. See Molins PLC v. Textron, Inc., 48 F.3d 1172, 1180 n.10, 33 USPQ2d 1823, 1828 n.10 (Fed. Cir. 1995). In its Guidelines, the PTO has determined that the written description requirement can be met by "show[ing] that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with

a known or disclosed correlation between function and structure, or some combination of such characteristics." Guidelines, 66 Fed. Reg. at 1106. For example, the PTO would find compliance with § 112, ¶ 1, for a claim to an "isolated antibody capable of binding to antigen X," notwithstanding the functional definition of the antibody, in light of "the art-recognized method of making antibodies to fully characterized antigens, the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that *the antibody technology is well developed and mature.*" Synopsis of Application of Written Description Guidelines, at 60, available at http://www.uspto.gov/web/patents/guides.htm ("Application of Guidelines").

In this case there should be no issue since applicants are claiming a fusion of known proteins.

The Examiner asserted that applicants have not provided proper, or enough, functional language to provide structural information commonly possessed by all members of the genus. The Examiner further asserted that, at the time of the invention, "there was, and still is, not way to predict or divine the enzyme-encoding sequences form sources other than those cited in the specification."

This is simply incorrect. Enzymes are defined by their function - their ability to catalyze a reaction based on one or more appropriate substrates. Enzyme nomenclature has been around for many decades. Appropriate examples of the claimed enzymes are known, publicly available,

and applicants have actually reduced to practice representative examples. Nothing further is legally required.

Medline indicates that for each of the claimed classes of enzymes, the amino acid sequence and a cDNA encoding the enzyme are known from multiple sources, and provide evidence that not only is the function generally the same between enzymes of different sources, but that the degree of homology is such that the known and available genes can be used to isolate additional genes from other sources encoding the enzymes. Such homology is the driving force behind the use of, for example, DNA microarrays (see above).

Enzymatic Function

As discussed in previous response to the previously issued Office Actions, the applicants have clearly stated that the cited enzymes, E1 and E2, catalyze successive reactions in a polyhydroxyalkanoate biosynthetic pathway and are each selected from the group consisting of β-ketothiolases, acyl-CoA reductases, polyhydroxyalkanoate synthases, poly(3-hydroxybutyrate) synthases, phasins, enoyl-CoA hydratases, and beta-hydroxyacyl-ACP::coenzyme-A transferases. These enzymes are known and well characterized. Their sequences are available in public databases and their sources are cited in the specification. The reagents and methods required for the functional expression of the genes encoding specific polyhydroxyalkanoate biosynthetic pathway are more than adequately described in the specification. Actual working examples are provided in the specification. Each enzymatic function can be readily ascertained, as provided for *via* example in the specification. For example, see Table 1 of the specification,

wherein thiolase and reductase activities are measured; as well as the percent PHB of dry cell weight.

Rejection Under 35 U.S.C. § 103

Claims 1-3, 5 and 6 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,245,023 to Peoples *et al.* ("Peoples"), in view of *Trends Biotechnol.* 9:226-231 by Bulow ("Bulow"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Peoples

Peoples teaches the construction of polymerase fusions for the purpose of "altering the enzyme's specificity to create novel polymerases" (see column 23, lines 14-24). The protein fusions described in Peoples are directed to PHB polymerase and PHA polymerase genes. Such fusions would not catalyze successive reactions in a polyhydroxyalkanoate pathway, but ***alternative reactions - i.e., either addition of short chain or long chain substrate.*** The claimed composition is the fusion of two enzymes that catalyze successive reactions in a polyhydroxyalkanoate biosynthetic pathway (i.e. each enzymatic "domain" of the fusion recognizes their normal (cognate) substrate). Therefore, the fusion enzyme of Peoples does not provide an indication of the success of a fusion enzyme that catalyzes the successive PHA biosynthetic reactions.

Bulow

Bulow teaches the optimal length linkers, for the enzymes described therein, based upon the correct folding and accessibility of active sites in the recombinant enzymes. Bulow's statements relating to enzyme technology and its usefulness in the development of metabolic engineering are entirely prophetic (see entire last paragraph of Bulow). Such suggestions do not create an expectation of success without ***evidence*** suggesting the modification (i.e. fusion catalyzing **successive** reactions) would be successful (for example, see *In re O'Farrell*, 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988).

Summary

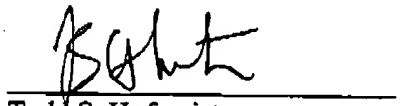
Peoples suggests the construction of a fusion between two polymerases (PHA and PHB) so that one could utilize more substrates **in the same reaction: the addition of monomer into the growing polymer chain.** Bulow teaches peptide linkers for use in making fusion proteins. The examiner has completely failed to provide evidence that one skilled in the art would have an expectation of success in combining two catalytically active enzymes, **using different substrates, one of which produces the substrate for the second enzyme,** in a single fusion protein.

Claim 4 was rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,245,023 to Peoples *et al.* ("Peoples"), in view of *Trends Biotechnol.* 9:226-231 by Bulow ("Bulow") as applied to claims 1-3, 5 and 6, and further in view of *J. Mol. Biol.* 211:943-958, 1990 by Argos ("Argos"). This rejection is respectfully traversed.

Applicants respectfully submit that in view of the foregoing discussion, as it relates to the rejection of claims 1-3, 5 and 6 under 35 U.S.C. § 103(a), the rejection of claim 4 is rendered moot.

Allowance of claims 1-6 is respectfully solicited.

Respectfully submitted,



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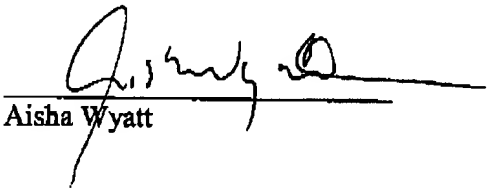
Certificate of Facsimile Transmission

I hereby certify that this Amendment and Response to Office Action, and any documents referred to as attached therein are being facsimile transmitted on this date, May 12, 2003, to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.



Aisha Wyatt

Date: May 12, 2003